

Effects of preparation method on the physicochemical characteristics and cell transfection efficiency of non-viral nanocarriers

A.C.N. Oliveira⁽¹⁾, J.P. Neves Silva⁽²⁾, P.J.G. Coutinho⁽²⁾, A. C. Gomes⁽¹⁾, O.P. Coutinho⁽¹⁾, M.E.C.D. Real Oliveira^(2,3)

⁽¹⁾ CBMA - Center of Molecular & Environmental Biology, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal

⁽²⁾ Center of Physics, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal

⁽³⁾ To whom correspondence should be addressed. E-mail: beta@fisica.uminho.pt

The success of gene therapy is decisively dependent on the development of effective carrier systems, able to compact and thus protect the genetic material as to avoid both *in vitro* and *in vivo* barriers for transgene delivery [1]. Given the problems of safety encountered with viral vectors, non-viral carrier systems are a safer alternative for the therapeutic delivery of genes [2]. Cationic liposomes/DNA (lipoplexes) have been widely used as non-viral vectors for cell transfection, with the neutral lipid (*helper*) of the liposome formulation playing a determinant role for the efficiency of this process. The lipoplex preparation method itself influences the structural properties of the produced lipoplex, affecting its final lipofection capability [3, 4]. In this work, we have studied the potential for cell transfection of a new liposomal formulation containing monoolein (MO) as *helper* lipid, using various preparation methods. Lipoplexes composed of pSV- β -galactosidase and dioctadecyldimethylammonium bromide (DODAB)/1-monooleoyl-rac-glycerol (MO) at (4:1) molar ratio and at cationic lipid/DNA ratio of 4.0 were tested. Ethidium Bromide (EtBr) exclusion assays, Dynamic Light Scattering (DLS), and Zeta Potential (ζ) were used to study plasmidic DNA complexation and physicochemical properties of the formed lipoplexes (their size and electrical charge). Transfection efficiency was also evaluated on 293T cells by determining the activity of β -galactosidase, the reporter gene. The results indicate that the lipoplexes' physicochemical properties are strongly dependent on the preparation method (one-step or multi-step complexation, at 25°C or 50°C), with resulting mean sizes varying from 350 to 1700 nm and superficial charge densities ranging from +9 mV to +30 mV. No clear correlation was found, however, between lipoplex physicochemical properties and observed cell transfection efficiencies, suggesting that other parameters, such as structure (degree of DNA condensation/compaction) of the complex and the mode of internalization by the cell may also weigh in on cell transfection. Nevertheless, it was possible to determine that lipoplex preparation methods can greatly affect DNA uptake by cells. Optimal nanotherapy requires the therapeutic molecule to be protected from degradation and reach its target cell and intracellular location. This work demonstrates that the MO-based system is a viable nanocarrier for plasmidic DNA and that the conditions to prepare the carriers for gene therapy application can be modulated to achieve maximal delivery efficiency.

[1] Edelstein M. L., Abedi M. R., Wixon J., Edelstein R. M. Gene therapy clinical trials worldwide 1989–2004 – an overview (2004) *The Journal of Gene Medicine* **6**: 597–602.

[2] Oliveira A.C., Ferraz M.P., Monteiro F.J. and Simões S., Cationic liposome–DNA complexes as gene delivery vectors: Development and behaviour towards bone-like cells, 2009, *Acta Biomaterialia*, **5**, 2142–2151.

[3] Ross, P.C. and S.W. Hui, *Lipoplex Size is a Major Determinant of In Vitro Lipofection Efficiency*. *Gene Therapy*, 1999. **6**: p. 651–659.

[4] Almofti, M.R., et al., *Lipoplex Size Determines Lipofection Efficiency With or Without Serum*. *Molecular Membrane Biology*, 2003. **20**: p. 35–43.